BIO RESCUE

BIOrescue:
A novel biorefinery concept for mushroom compost

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Deliverable 4.5
Mutant libraries of cellulases and hemicellulases

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Author: UNINA
Contributor: MetGen

PUBLISHABLE SUMMARY

This report describes the activities conducted in Task 4.4 “Novel enzyme development”, in particular cloning, recombinant expression and characterisation of two cellulases, Cel 1 and Cel 2, in order to select the best enzyme to be subjected to directed evolution aimed at improving its properties. The production costs of cellulases represents the main contributor to the cost of the overall process of lignocellulosic biomasses’ industrial conversion into second-generation biofuels or other high-added-value bioproducts. Furthermore, huge amounts of enzymes are required for the lignocellulose hydrolysis at an industrial scale, making the production of biochemicals uncompetitive. Therefore, the objective of the activities described in this report were to obtain cellulases variants in order to improve the hydrolytic yield of the parental enzyme and thus contribute to the cost reduction of biomass conversion, making the process more competitive.

To achieve this objective, twenty-four bacterial strains belonging to Actinobacteria were previously identified to select microorganisms producing biocatalysts able to hydrolyse pretreated lignocellulosic biomasses. These Actinobacteria strains were then screened for their cellulase activity production in liquid growth medium and the extracellular enzymatic mixture from the best cellulase producer was successfully applied to the hydrolysis of the biomasses giant reed, poplar and switchgrass. This strain was therefore selected to identify the proteins responsible for the cellulase activity. Several proteins were confidently identified in different *Streptomyces spp*., 8 of which belonging to the class of Carbohydrate active enzymes. In the framework of the BIOrescue project, the two new cellulases Cel1 and Cel2 were subjected to cloning and recombinant expression in order to select the best enzyme developing improved variants for conversion of spent mushroom substrate (SMS) through directed evolution. The genes coding for Cel1 and Cel2 were cloned into two different expression vectors and expressed in *Escherichia coli*. The two recombinant proteins were subjected to functional characterisation. Cel 2 exhibited a relevant thermoresitance and pH resistance, it was therefore selected to be subjected to the directed evolution. The strategy for diversity generation by directed evolution was set up. This strategy was validated by applying it

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to generate and screen 5000 Cel2 mutants demonstrating that 6 of these mutants show higher activity than wild type Cel2.

This strategy was also applied to obtain another set of 25,000 transformants, allowing us to achieve the expected final goal of this deliverable, namely to generate a total of 30,000 mutants.

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